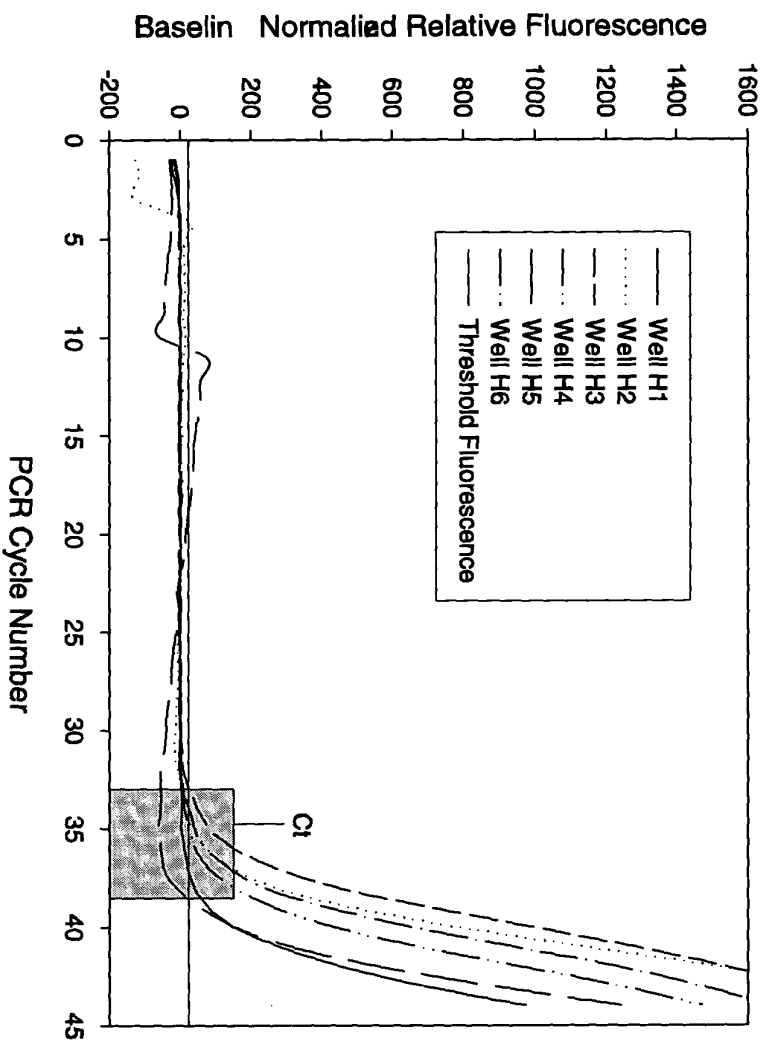
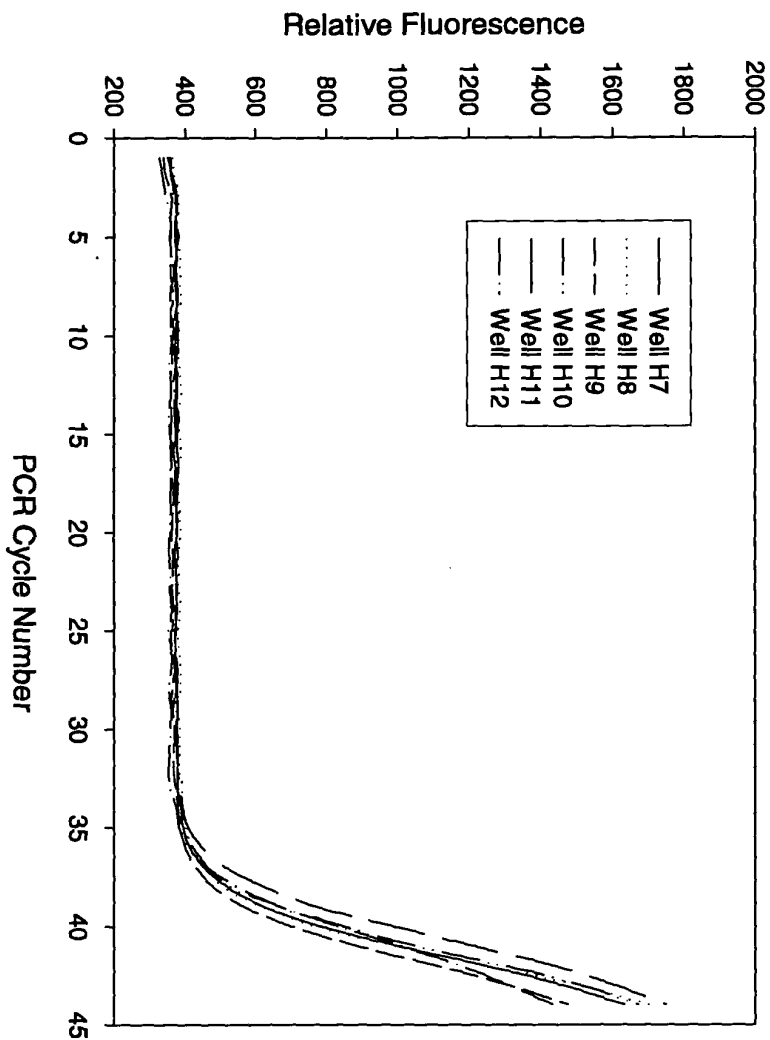


**Figure 1.** Effect of surfactant foaming on fluorescent signal of real-time PCR of low copy template. Plot of raw relative fluorescence readings collected at each cycle during PCR of 20 copies  $\beta$ -actin template, amplified in the presence of SYBR Green I, for 6 representative reactions from a 48-reaction set. Perturbation to basal fluorescence is evident in plots for PCRs from well H2 and H5.

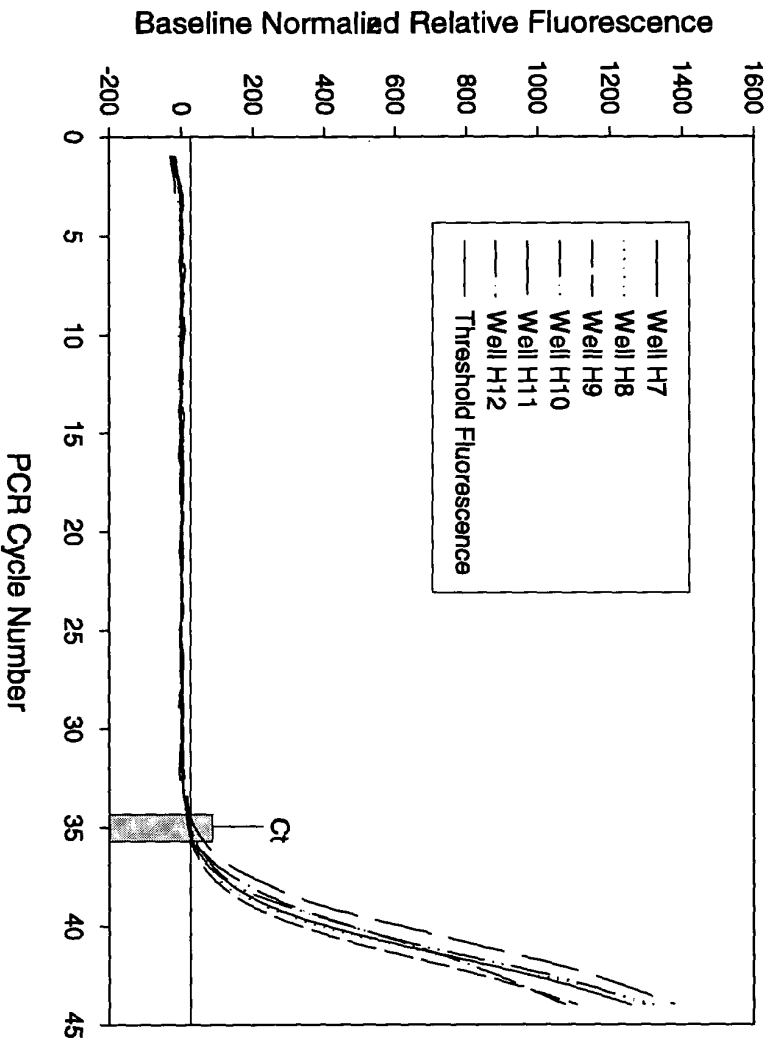


**Figure 2.** Effect of surfactant foaming on threshold cycle (Ct) determination in real-time PCR of low copy template. Plot of baseline normalized relative fluorescence readings collected at each cycle during PCR of 20 copies  $\beta$ -actin template, amplified in the presence of SYBR Green I, for 6 representative reactions from a 48 reaction set. Perturbation to basal fluorescence in PCRs for wells H2 and H5 results in distortion of baseline and aberrant determination of threshold cycle (Ct) with poor precision. Range of Ct values indicated by grey box.

Atty. Dkt. No. 38266-0009  
Inventors: Ayoub Rashtchian et al.  
Title: IMPROVED COMPOSITIONS  
FOR IN VITRO AMPLIFICATION OF  
NUCLEIC ACIDS  
Filed: August 5, 2003



**Figure 3.** Control of surfactant foaming by anti-foam enables stable basal fluorescence during real-time PCR of low copy template. Plot of raw relative fluorescence readings collected at each cycle during PCR of 20 copies  $\beta$ -actin template, amplified in the presence of SYBR Green I and 0.003% Dow 1520-US anti-foam, for 6 representative reactions (wells H7 – H12) from a 48 reaction set.



**Figure 4.** Anti-foam improves precision of Ct results for real-time PCR of low copy template. Plot of baseline normalized relative fluorescence readings collected at each cycle during PCR of 20 copies  $\beta$ -actin template, amplified in the presence of SYBR Green I and 0.003% Dow 1520-US anti-foam, for 6 representative reactions (wells H7 – H12) from a 48 reaction set. Range of Ct values indicated by grey box.